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Synthesis of hydroxylated PCB metabolites with the Suzuki-coupling

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Abstract

An improved synthesis of hydroxylated polychlorinated biphenyls (PCBs) which are structurally related to the major hydroxy PCB congeners identified in human plasma is described. The coupling of (chlorinated) aryl boronic acids with bromochloro anisoles using the standard conditions of the Suzuki coupling gave the desired hydroxylated PCB metabolites in good to excellent yields. The approach offers the advantage of high selectivity and good yields compared to conventional methods such as the Cadogan reaction and allows the use of less toxic starting materials.

Keywords: PCB; Suzuki-coupling; Environmental contaminants

1. Introduction

Polychlorinated biphenyls (PCBs) are industrial chemicals that were used as dielectrics, cooling fluids, lubricants and flame retardants. Unfortunately, PCBs have escaped into the environment where they persist and accumulate in animal and human tissues, including human adipose tissue, serum and breast milk (Hansen, 1987; Hansen, 1994; Hansen, 1999). The production of PCBs have been banned in industrialized countries and environmental levels of these compounds are decreasing in many locations. PCBs however continue to persist and to bioaccumulate, characteristics that still raise concerns about the long-term health effects of these chemicals. Many mechanisms of adverse effects are still poorly understood.

PCBs are metabolized in vivo to hydroxy- and sulfur-containing metabolites (for a general review see: Letcher et al., 2000). Hydroxylation proceeds primarily at the meta and para position either via an arene oxide, involving NIH shift, or by direct insertion of a hydroxyl group. Monohydroxy, as well as *ortho*-dihydroxy (catechol) and *para*-dihydroxy (hydroquinone), metabolites of PCBs have been found in numerous species, including rats, guinea pig and seals (Bergman et al., 1994; McLean et al., 1996; Ariyoshi et al., 1997; Haraguchi et al., 1998). Several monohydroxy metabolites of higher chlorinated biphenyls have also been found in human serum (Bergman et al., 1994). Hydroxylated PCBs have been shown to inhibit mitochondrial oxidative phosphorylation (Narasimhan et al., 1991), thyroid hormone sulfation (Schuur et al., 1998a,b,c) as well as estrogen sulfotransferase (Kester et al., 2000), to affect thyroxine (T4) levels (Sinjari and Darnerud, 1998) and to exhibit estrogenic or antiestrogenic activity (Korach et al., 1987; Bergeron et al., 1994; Fielden et al., 1997; Moore et al., 1997; Vakharia and Gierthy, 2000). Also of major concern is the developmental effect in pregnant or fetal mammals (Morse et al., 1995; Sinjari and Darnerud, 1998). Still many questions regarding their biological and toxic effects remain, largely because these compounds are difficult to synthesize in large quantities for biological studies.

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The Cadogan coupling (Cadogan et al., 1962) is the classic approach to synthesize hydroxylated PCBs (Mannila et al., 1994; Bergman et al., 1995; Safe et al., 1995), but the low yields and poor regioselectivity of this reaction are major drawbacks and make the preparation of large (gram) quantities difficult and tedious. Ullmann and crossed Ullmann reactions are also possible approaches to hydroxylated PCBs, but crossed Ullmann reactions are low-yielding reactions and significant amounts of highly toxic dibenzofuran byproducts may be formed (Moron et al., 1973; Goldstein et al., 1978). Furthermore, the synthesis of suitable starting materials often requires a multi-step synthesis (Waller et al., 1999). Finally, the direct functionalization of suitable PCB congeners via an aniline derivative is a low yield reaction (Hutzinger et al., 1983; Waller et al., 1999).

We recently published the straightforward synthesis of several PCB congeners (Lehmler and Robertson, 2001) and various (di-)methoxylated and (di-)hydroxylated PCB metabolites via the Suzuki coupling (Bauer et al., 1995; McLean et al., 1996). This cross-coupling reaction between an aryl boronic acid and an aryl bromide (Miyaura and Suzuki, 1995; Stanforth, 1998; Suzuki, 1999) was conducted in the presence of tetrakis(triphenylphosphine)palladium (0) and aqueous sodium carbonate in toluene/ethanol at 80°C for 12 h. Subsequent demethylation of the methoxylated PCB using boron tribromide (BBr_3) yielded chlorinated hydroxy or dihydroxy biphenyls. Herein we report the application of this approach to the synthesis of several 2- and 4-hydroxylated PCB congeners, which are structurally related to the major hydroxy PCB congeners identified in human plasma (Bergman et al., 1994), thus making these compounds available in gram quantities for biological and toxicological studies.

2. Materials and methods

All hydroxylated PCB congeners were characterized by ^1H and ^{13}CNMR, FT-IR and GC/MS spectroscopy. The IR spectra were obtained using a Nicolet Magna-IR 560 Spectrometer E.S.P. The ^1H and ^{13}CNMR spectra were recorded on a Varian Gemini 200 or a Varian VXR-400S NMR Spectrometer by using $CDCl_3$ (Cambridge Isotope Laboratories, Andover, MA) as solvent and TMS (tetramethylsilane) as internal standard. Combustion analysis of all methoxylated and hydroxylated PCB congeners was performed by Atlantic Microlab (Atlanta, GA). The purity of all methoxy compounds was analyzed with a Hewlett Packard 5890 A Gas Chromatograph equipped with a HP-1 (Methyl Silicone Gum) column (Hewlett Packard, Avondale, PA) and determined based on relative peak area. The following conditions were used for the gas chromatographic analysis: injector: 255°C, detector (FID): 300°C, starting temperature: 40°C, final temperature: 245°C, heating rate: 10°/min. GC/MS analysis of both methoxylated and hydroxylated PCBs were performed by the Mass Spectrometry Facility of the University of Kentucky (Lexington, KY). The melting points were determined on a MEL-TEMP apparatus and are uncorrected. The analytical data of all compounds is in agreement with their proposed structure as well as literature data (Safe and Hutzinger, 1972; Welti and Sissons, 1972; Wilson, 1975; Yanagisawa et al., 1986; Yanagisawa et al., 1987). *Caution*: PCBs are reasonably anticipated to be human carcinogens and should therefore be handled in an appropriate manner. All phenols and anisoles have a high vapor pressure and should be handled in a fume hood.

2.1. 2,6-Dichloro-4-bromophenol (1a)

A solution of 3.7 ml (11.3 g, 61.3 mmol) bromine in glacial acetic acid was added to a warm solution of 10.0 g (61.3 mmol) 2,6-dichlorophenol in glacial acetic acid. The glacial acetic acid was removed under reduced pressure. The oily residue solidified at room temperature and was recrystallized twice from *n*-hexanes to yield 4.87 g (33%) of the 2,6-dichloro-4-bromophenol **1a** as white needels.

m.p. = 60–61°C (Lit.: 66.5°C or 68°C). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.81 (s, OH, 1H), 7.38 (s, $C_{Ar}H$, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 111.70, 121.90, 130.78 ($\underline{C}_{Ar}H$, 2C), 147.30. IR [cm^{-1}]: 3372, 3074, 1568, 1470, 1390, 1360, 1330, 1272, 1226, 1175, 856, 810, 794, 708, 688. MS m/z (relative intensity, %): 240 (62, $C_6H_3Cl_2BrO^{\cdot+}$), 204 (3, M–HCl), 176 (9, M–HCl–CO), 97 (21).

2.2. 2,3,6-Trichloro-4-bromophenol (1b) (Kohn and Fink, 1930)

Similar to the above procedure, a solution of 1.7 ml (4.0 g, 25.0 mmol) bromine in glacial acetic acid was added to a warm solution of 5.0 g (25.0 mmol) 2,3,6-tichlorophenol in glacial acetic acid. A brown solid crystallized from the cold reaction mixture. Filtration and a second recrystallization from glacial acidic acid yielded 4.34 g (67%) of 2,3,6-trichloro-4-bromophenol **1b** as white solid.

m.p. = 65°C (Lit.: 80°C). ^{1}H-NMR ($CDCl_3$, 200 MHz) $\delta \sim 3.19$ (s, OH, 1H), 7.78 (s, $C_{Ar}H$, 1H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 112.67, 121.88, 122.85, 132.22 ($\underline{C}_{Ar}H$, 1C), 132.65, 150.87. IR [cm^{-1}]: 3489, 3085, 1550, 1444, 1385, 1286, 1204, 1165, 867, 711. MS m/z (relative intensity, %): 274 (51, $C_6H_2Cl_3BrO^{\cdot+}$), 131 (34), 96 (23), 61 (28).

2.3. 4-Bromo-2,6-dichloroanisole (2a) (Kohn and Sussmann, 1925)

4.87 g (20.3 mmol) 2,6-dichloro-4-bromophenol was dissolved in an aqueous sodium hydroxide solution (1.0 g

(25.4 mmol) sodium hydroxide in 9 ml water). 1.9 ml (20.3 mmol) dimethylsulfate was added slowly to this solution and the formation of a white precipitate was observed. After the addition was complete, the reaction mixture was heated under reflux for 30 min to destroy excess dimethylsulfate. The reaction mixture was extracted with diethyl ether (3×10 ml), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure to yield 2.67 g (52%) of a white solid. Recrystallization from *n*-hexanes yielded a white solid with a purity of >99%.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.85 (s, $OC\underline{H}_3$, 3H), 7.42 (s, $C_{Ar}H$, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 60.77 ($O\underline{C}H_3$), 116.43, 130.33, 131.54 ($\underline{C}_{Ar}H$, 2C), 151,83. IR [cm^{-1}]: 3074, 2949, 1567, 1551, 1466 1416, 1380, 1366, 1258, 985, 859, 828, 807, 790, 747, 671, 571. MS m/z (relative intensity, %): 254 (51, $C_7H_5Cl_2BrO^{\cdot+}$), 239 (45, M–CH_3), 211 (19, M–CH_3–CO), 97 (23), 74 (13), 61 (24), 43 (100).

2.4. 4-Bromo-2,3,6-trichloroanisole (2b) (Kohn and Fink, 1930)

Following the procedure given above, methylation of 4.34 g (15.7 mmol) of 4-bromo-2,3,6-trichlorophenol with 1.50 ml (1.98 g, 15.7 mmol) dimethylsulfate gave the corresponding anisole **2b** in 27% (1.2 g) yield after recrystallization from ethanol.

m.p. = 49–50°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.90 (s, $OC\underline{H}_3$, 3H), 7.62 (s, $C_{Ar}H$, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 60.76 ($O\underline{C}H_3$), 118.07, 128.00, 129.96, 131.86 (CH), 132.87, 152.79. IR [cm^{-1}]: 3065, 2998, 2942, 1453, 1409, 1349, 1253, 1006, 866, 816, 696. MS m/z (relative intensity, %): 288 (48, $C_7H_4Cl_3BrO^{\cdot+}$), 273 (52, M–CH_3), 245 (55, M–CH_3–CO), 131 (58), 108 (27), 96 (46), 61 (46).

2.5. 4-Bromo-2-chloroanisole (2c) (Li et al., 1996)

Following the procedure given above, methylation of 14.47 g (69.7 mmol) of 4-bromo-2-chlorophenol with 6.60 ml (8.80 g, 69.7 mmol) dimethylsulfate gave the corresponding anisole in 88% (13.6 g). The crude product was used directly without further purification.

m.p. = 50–54°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.84 (s, $OC\underline{H}_3$, 3H), 6.75 (d, J_{meta} = 2.6 Hz, H-3), 7.28 (d, $J_{ortho} = 8.8$ Hz, d, $J_{meta} = 2.6$ Hz, H-5), 7.45 (d, $J_{ortho} = 8.8$ Hz, H-6). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 56.02 ($O\underline{C}H_3$), 112.28, 113.11, 123.39, 130.37, 132.42, 154.18. IR [cm^{-1}]: 3088, 3066, 3023, 2975, 2937, 2888, 2837, 1482, 1461, 1293, 1265, 1253, 1064, 1019, 875, 806, 709. MS m/z (relative intensity, %): 220 (87, $C_7H_6ClBrO^{\cdot+}$), 205 (70, M–CH_3), 177 (53, M–CH_3–CO), 126 (13), 110 (7), 98 (13), 75 (25), 63 (60), 50 (13).

2.6. 2-Bromo-4-chloroanisole (2d) (Hussey and Wilk, 1950)

Following the procedure given above, methylation of 25.0 g (121.0 mmol) of 2-bromo-4-chlorophenol with 11.4 ml (15.2 g, 121.0 mmol) dimethylsulfate gave the corresponding anisole **2d** in 98% (26.4 g) as a yellow oil. The crude product was used directly without further purification.

^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.85 (s, $OC\underline{H}_3$, 3H), 6.79 (d, $J_{ortho} = 8.8$ Hz, H-3), 7.22 (d, $J_{ortho} = 8.8$ Hz, d, $J_{meta} = 2.6$ Hz, H-5), 7.51 (d, $J_{meta} = 2.6$ Hz, $H-6$). $^{13}C-NMR$($CDCl_3$, 50 MHz) δ 56.38 ($O\underline{C}H_3$), 112.07, 112.48, 125.89, 128.20, 132.73, 154.71. IR [cm^{-1}]: 3094, 3070, 3008, 2964, 2936, 2902, 2838, 1480, 1461, 1438, 1289, 1263, 1249, 1052, 803, 689. MS m/z (relative intensity, %): 220 (79, $C_7H_6ClBrO^{\cdot+}$), 205 (58, M–CH_3), 177 (40, M–CH_3–CO), 126 (12), 111 (9), 98 (8), 75 (21), 63 (36), 50 (11).

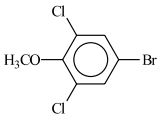
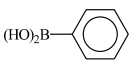
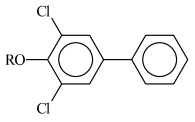
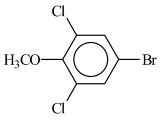
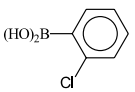
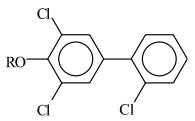
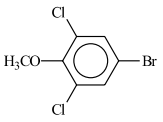
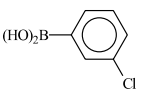
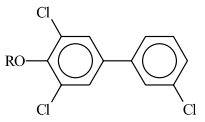
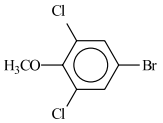
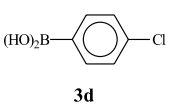
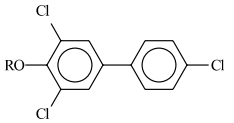
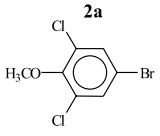
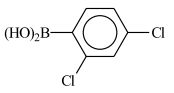
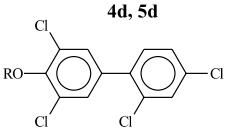
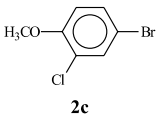
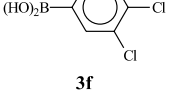
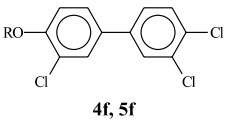
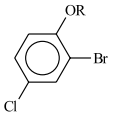
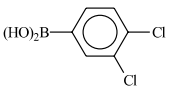
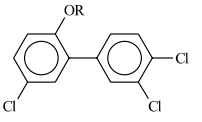
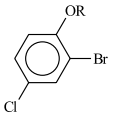
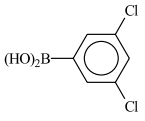
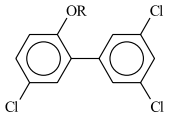
2.7. General procedure for the Suzuki cross-coupling (Bauer et al., 1995; McLean et al., 1996)

Sodium carbonate (5 ml, 2 M aq.) was added to a solution of an anisole **2** (5 mmol) and $Pd(PPh_3)_4$ (0.18 mg) in toluene (20 ml). A solution of a chlorobenzene boronic acid **3** (5 mmol) in ethanol (10 ml) was added slowly to the solution of the bromo anisole under a nitrogen atmosphere. The reaction mixture was maintained at 80°C for 12–16 h. Hydrogen peroxide (0.5 ml, 30%) was added slowly to the warm reaction mixture to destroy unreacted boronic acid. The mixture was stirred at room temperature for additional 4 h and diluted with diethyl ether (30 ml). The reaction mixture was extracted once with NaOH (10 ml, 2 M aq.) and three times with water (20 ml). The organic phase was dried over $MgSO_4$ and the solvents were removed under reduced pressure. Column chromatography (glass column, 300 mm × 25 mm i.d.) over Alumina (Alumina Adsorption 80–200 mesh, Fisher Scientific, Pittsburg, PA) with *n*-hexanes as eluent yielded the desired methoxylated PCB congener **5** or **7**. The yields are summarized in Table 1.

2.8. 3,5-Dichloro-4-methoxybiphenyl (4a)

m.p. = 50–51°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.93 (s, OCH_3, 3H), 7.30–7.7.58 (m, 7H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 60.77 ($O\underline{C}H_3$), 126.83, 127.36, 128.07, 128.95, 129.54, 138.21, 138.63, 151.41. IR [cm^{-1}]: 3087, 3067, 3028, 3008, 2982, 2937, 2872, 2835, 1545, 1473, 1428, 1376, 1297, 1248, 1203, 1085, 1076, 1062, 992, 868, 805, 755, 695. MS m/z (relative intensity, %): 252 (83, $C_{13}H_{10}Cl_2O^{\cdot+}$), 237 (100, M-$CH_3$), 209 (39, M–CO–$CH_3$), 173 (17, M–CO–$CH_3$–HCl), 152 (14), 139 (36, M–CO–CH_3–Cl_2). Elemental analysis calcd. for $C_{13}H_{10}Cl_2O$: C 61.69, H 3.98; found: C 61.54, H 3.99.

Table 1
Synthesis of 4-hydroxy PCB metabolites

Bromoanisole	Boronic Acid	Methoxy/Hydroxy Biphenyl	Yield (R = Me) [%]	Yield (R = H) [%]
 **2a**	 **3a**	 **4a, 5a**	49	69
 **2a**	 **3b**	 **4b, 5b**	92	83
 **2a**	 **3c**	 **4c, 5c**	76	95
 **2a**	 **3d**	 **4d, 5d**	87	83
 **2a**	 **3e**	 **4e, 5e**	62	72
 **2c**	 **3f**	 **4f, 5f**	58	57
 **2d**	 **3f**	 **6a, 7a**	91	69
 **2d**	 **3g**	 **6b, 7b**	66	43

2.9. 2′,3,5-Trichloro-4-methoxybiphenyl (4b)

m.p. = 79–81°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.94 (s, OCH_3, 3H), 7.23–7.31 (m, 3H), 7.36 (s, H-2,6), 7.40–7.47 (m, 1H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 60.64 ($O\underline{C}H_3$), 126.95, 128.80, 129.26, 129.78 (2 × CH), 130.02, 130.94, 132.19, 136.44, 137.45, 151.61. IR [cm^{-1}]: 3060, 3007, 2983, 2939, 2834, 1546, 1489, 1465, 1426, 1374, 1303, 1261, 1241, 1077, 1039, 994, 881, 808, 754. MS m/z (relative intensity, %): 286 (100, $C_{13}H_9Cl_3O^{\cdot+}$), 271 (72, M–CH_3), 243 (30, M–CO–CH_3), 173 (40, M–CO–CH_3–Cl_2). Elemental analysis calcd. for $C_{13}H_9Cl_3O$: C 54.30, H 3.15; found: C 54.26, H 3.09.

2.10. 3,3′,5-Trichloro-4-methoxybiphenyl (4c)

m.p. = 73–75°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.90 (s, OCH_3, 3H), 7.26–7.30 (m, 3H), 7.37–7.42 (m, 1H), 7.39 (s, H-2,6). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 60.64 ($O\underline{C}H_3$), 124.8, 126.77, 127.17 (2 × CH), 127.96, 129.64, 130.06, 134.77, 136.87, 139.75, 151.81. IR [cm^{-1}]: 3071, 2943, 1545, 1492, 1467, 1424, 1372, 1289, 1254, 1213, 1170, 1103, 1088, 1076, 1063, 985, 864, 804, 781, 736, 692, 682. MS m/z (relative intensity, %): 286 (100, $C_{13}H_9Cl_3O^{\cdot+}$), 271 (84, M–CH_3), 243 (32, M–CO–CH_3), 173 (40, M–CO–CH_3–Cl_2). Elemental analysis calcd. for $C_{13}H_9Cl_3O$: C 54.30, H 3.15; found: C 54.55, H 3.16.

2.11. 3,4′,5-Trichloro-4-methoxybiphenyl (4d)

m.p. = 82–83°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.94 (s, OCH_3, 3H), 7.42 (s, H-2′,3′,5′,6′), 7.46 (s, H-2,6). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 60.81 ($O\underline{C}H_3$), 127.24 (CH), 128.09 (2 CH), 128.16 (CH), 129.75, 134.29, 136.67, 137.36, 151.72. IR [cm^{-1}]: 2951, 2834, 1475, 1425, 1376, 1295, 1251, 1093, 1014, 990, 824, 820, 803, 758, 509. MS m/z (relative intensity, %): 286 (97, $C_{13}H_9Cl_3O^{\cdot+}$), 271 (100, M–CH_3), 243 (31, M–CO–CH_3), 173 (40, M–CO–CH_3–Cl_2). Elemental analysis calcd. for $C_{13}H_9Cl_3O$: C 54.30, H 3.15; found: C 54.47, H 3.13.

2.12. 2′,3,4′,5-Tetrachloro-4-methoxybiphenyl (4e)

m.p. = 97°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.97 (s, OCH_3, 3H), 7.23 (d, J_{ortho} = 8.6 Hz, H-6′), 7.32 (d, J_{ortho} = 8.6 Hz, d, J_{meta} = 2.2 Hz, H-5′), 7.36 (s, H-2,6), 7.50 (d, J_{meta} = 2.2 Hz, H-3′). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 60.78 ($O\underline{C}H_3$), 127.37, 129.08, 129.74 (2 CH), 129.90, 131.75, 133.08, 134.60, 135.38, 136.10, 151.95. IR [cm^{-1}]: 2941, 2838, 1465, 1428, 1384, 1361, 1297, 1105, 1077, 991, 862, 820, 806, 795. MS m/z (relative intensity, %): 320 (78, $C_{13}H_8Cl_4O^{\cdot+}$), 305 (68, M–CH_3), 277 (30, M–CO–CH_3), 207 (42, M–CO–CH_3–Cl_2). Elemental analysis calcd. for $C_{13}H_8Cl_4O$: C 48.49, H 2.50; found: C 48.44, H 2.54.

2.13. 3,3′,4′-Trichloro-4-methoxybiphenyl (4f)

m.p. = 110–111°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 3.92 (s, OCH_3, 3H), 6.96 (d, J_{ortho} = 8.4 Hz, H-5), 7.30 (d, J_{ortho} = 8.4 Hz, d, J_{meta} = 2.2 Hz, H-6′), 7.36 (d, J_{ortho} = 8.4 Hz, d, J_{meta} = 2.2 Hz, H-6), 7.44 (d, J_{ortho} = 8.4 Hz, H-5′), 7.52 (d, J_{meta} = 2.2 Hz, H-2*), 7.56 (d, J_{meta} = 2.2 Hz, H-2′*). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 56.17 ($O\underline{C}H_3$), 112.24, 123.00, 125.76, 126.07, 128.32, 128.52, 130.65, 131.22, 131.91, 132.83, 139.40, 154.94. MS m/z (relative intensity, %): 286 (100, $C_{13}H_9Cl_3O^{\cdot+}$), 271 (65, M–CH_3), 243 (39, M–CO–CH_3), 173 (35, M–CO–CH_3–Cl_2). IR [cm^{-1}]: 3055, 3016, 2956, 2939, 2905, 2836, 1540, 1466, 1440, 1292, 1255, 1065, 1026, 809. Elemental analysis calcd. for $C_{13}H_9Cl_3O$: C 54.30, H 3.15; found: C 54.41, H 3.16.

2.14. 3′,5,4′-Trichloro-2-methoxybiphenyl (6a)

m.p. = 74–76°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.80 (s, OCH_3, 3H), 6.90 (d, J = 8.4 Hz, H-3), 7.24 (d, J = 2.4 Hz, H-6), 7.30 (d, J = 8.4 Hz, d, J = 2.4 Hz, H-4), 7.33 (d, J = 8.4 Hz, d, J = 2.4 Hz, H-6′), 7.47 (d, J = 8.4 Hz, H-5′), 7.60 (d, J = 2.4 Hz, H-2′). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 55.73 ($O\underline{C}H_3$), 112.42, 125.70, 128.66, 128.86, 129.41, 129.88, 130.00, 131.10, 131.36, 131.98, 137.01, 154.81. MS m/z (relative intensity, %): 286 (67, $C_{13}H_9Cl_3O^{\cdot+}$), 273 (3, M–CH_3), 251 (3, M–Cl), 236 (100, M–Cl–CH_3), 173 (21). IR [cm^{-1}]: 3026, 2971, 2946, 2838, 1492, 1466, 1250, 1235, 1021, 812, 805. Elemental analysis calcd. for $C_{13}H_9Cl_3O$: C 54.30, H 3.15; found: C 54.47, H 3.33.

2.15. 3′,5,5′-Trichloro-2-methoxybiphenyl (6b)

m.p. = 97–98°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.79 (s, OCH_3, 3H), 6.88 (d, J = 8.0 Hz, H-3), 7.22 (d, J = 2.4 Hz, H-6), 7.28 (d, J = 8.0 Hz, d, J = 2.4 Hz, H-4), 7.31 (t, J = 2.0 Hz, H-4′), 7.36 (d, J = 2.0 Hz, H-2′,6′). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 55.88 ($O\underline{C}H_3$), 112.49, 125.82, 127.37, 127.85 (2 × C), 129.21, 129.32, 130.20, 134.50, 139.99, 154.89. MS m/z (relative intensity, %): 286 (70, $C_{13}H_9Cl_3O^{\cdot+}$), 251 (9, M–Cl), 236 (100, M–Cl–CH_3), 173 (27). IR [cm^{-1}]: 3080, 2962, 1556, 1492, 1432, 1417, 1373, 1264, 1231, 1181, 1142, 1101, 1060, 1032, 850, 807, 799, 683, 654. Elemental analysis calcd. for $C_{13}H_9Cl_3O$: C 54.30, H 3.15; found: C 54.50, H 3.22.

2.16. General procedure for the deprotection (Bauer et al., 1995; McLean et al., 1996)

Borontribromide (1 eq., 1 M in *n*-hexanes) was added to a solution of the methoxy PCB (1 eq.) in anhydrous dichloromethane (20 ml) under a nitrogen atmosphere. The reaction mixture was stirred at room temprature for 14–16 h, cooled with ice/salt and hydrolized with an equal volume of icecold water. The aqueous phase was extracted with dichloromethane (2 × 10 ml). The combined organic layers were washed with water (3 × 10 ml), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Column chromatography (glass column, 300 mm × 25 mm i.d.) over silica gel with *n*-hexanes/ethylacetate (gradient 100% to 90% *n*-hexanes) yielded the desired hydroxylated PCB congener. The yields are summarized in Table 1.

2.17. 3,5-Dichloro-4-hydroxybiphenyl (5a)

m.p. = 64°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 5.81 (s, –OH, 1H), 7.24–7.47 (m, ArH, 7H). ^{13}C-NMR ($CDCl_3$,

50 MHz) δ 121.38, 126.66, 126.80, 127.76, 128.96, 134.96, 138.37, 147.03. MS m/z (relative intensity, %): 238 (100, $C_{12}H_8Cl_2O^{\cdot+}$), 139 (94, M-99). IR [cm^{-1}]: 3411 (ν (OH)), 1474, 1293, 1236, 1180, 760, 697. Elemental analysis calcd. for $C_{12}H_8Cl_2O$: C 60.28, H 3.37; found: C 60.42, H 3.38.

2.18. 2′,3,5-Trichloro-4-hydroxybiphenyl (5b)

m.p. = 98°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 5.94 (br s, –OH, 1H), 7.24–7.32 (m, ArH, 3H), 7.37 (s, ArH, 2,6-H), 7.42–7.52 (m, ArH, 1H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 120.61, 126.99, 129.12, 129.22, 130.06, 131.06, 132.38, 132.68, 137.63, 147.25. IR [cm^{-1}]: 3431 (ν (OH)), 1573, 1562, 1496, 1464, 1439, 1394, 1342, 1301, 1234, 1179, 1035, 875, 810, 763, 744. MS m/z (relative intensity, %): 272 (100, $C_{12}H_7Cl_3O^{\cdot+}$), 173 (76, M-99). Elemental analysis calcd. for $C_{12}H_7Cl_3O$: C 52.69, H 2.58; found: C 52.85, H 2.59.

2.19. 3,3′,5-Trichloro-4-hydroxybiphenyl (5c)

m.p. = 107–109°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 5.87 (br s, –OH, 1H), 7.26–7.36 (m, ArH, 3-H), 7.43 (s, ArH, 2,6-H), 7.41–7.47 (m, ArH, 1-H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 121.60, 124.81, 126.81, 127.80, 130.19, 133.48, 134.94, 140.17, 147.58. IR [cm^{-1}]: 3488 (ν (OH)), 1463, 1328, 1234, 1173, 862, 778, 691. MS m/z (relative intensity, %): MS m/z (relative intensity, %): 272 (100, $C_{12}H_7Cl_3O^{\cdot+}$), 173 (59, M-99); identical with MS of 2′,3,5-trichloro-4-hydroxybiphenyl. Elemental analysis calcd. for $C_{12}H_7Cl_3O$: C 52.69, H 2.58; found: C 52.83, H 2.62.

2.20. 3,4′,5-Trichloro-4-hydroxybiphenyl (5d)

m.p. = 147°C. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 5.91 (br s, –OH, 1H), 7.35–7.50 (m, ArH, 2′,3′,5′,6′-H), 7.45 (s, ArH, 2,6-H). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 121.53, 126.66, 127.90, 129.13, 133.67, 133.92, 136.82, 147.31. IR [cm^{-1}]: 3431 (ν (OH)), 1496, 1464, 1439, 1394, 1342, 1301, 1271, 1267, 1234, 1179, 1128, 1035, 875, 810, 763, 744, 716, 685. MS m/z (relative intensity, %): 272 (100, $C_{12}H_7Cl_3O^{\cdot+}$), 173 (76, M-99); identical with MS of 2′,3,5-trichloro-4-hydroxybiphenyl. Elemental analysis calcd. for $C_{12}H_7Cl_3O$: C 52.69, H 2.58; found: C 52.71, H 2.60.

2.21. 3,2′,4′,5-Tetrachloro-4-hydroxybiphenyl (5e)

m.p. = 121–122°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.95 (br s, –OH, 1H), 7.22 (d, J_{ortho} = 8.2 Hz, H-6′), 7.30 (d, J_{ortho} = 8.2 Hz, d, J_{meta} = 2.1 Hz, H-5′), 7.33 (s, H-2,6), 7.48 (d, J_{meta} = 2.1 Hz, H-3′). ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 120.86, 127.42, 129.21 (2 × C), 129.94, 131.63, 131.87, 133.24, 134.46, 136.28, 147.63. IR [cm^{-1}]: 3442 (ν (OH)), 1591, 1572, 1463, 1407, 1340, 1298, 1255, 1231, 1178, 1109, 872, 807, 717. MS m/z (relative intensity, %): 306 (77, $C_{12}H_6Cl_4O^{\cdot+}$), 207 (44, M-99). Elemental analysis calcd. for $C_{12}H_6Cl_4O$: C 46.80, H 1.96; found: C 46.73, H 1.98.

2.22. 3,3′,4′-Trichloro-4-hydroxybiphenyl (5f)

m.p. = 90–91°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.65 (br s, –OH, 1H), 7.08 (d, J = 8.4 Hz, H-5), 7.32 (d, J = 8.4 Hz, d, J = 2.4 Hz, H-6′), 7.35 (d, J = 8.4 Hz, d, J = 2.4 Hz, H-6), 7.47 (d, J = 8.4 Hz, H-5′), 7.50 (d, J = 2.4 Hz, H-2), 7.58 (d, J = 2.4 Hz, H-2′). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 120.47, 125.84, 126.97, 127.33, 128.44, 130.72, 131.36, 132.35, 132.91, 139.47 (C-1′), 151.36 (C-1). MS m/z (relative intensity, %): 272 (86, $C_{12}H_7Cl_3O^{\cdot+}$), 202 (15, M–Cl_2), 173 (100, M-99). IR [cm^{-1}]: 3453 (ν (OH)), 1467, 1182, 810, 801. Elemental analysis calcd. for $C_{12}H_7Cl_3O$: C 52.69, H 2.58; found: C 52.79, H 2.63.

2.23. 3′,5,4′-Trichloro-2-hydroxybiphenyl (7a)

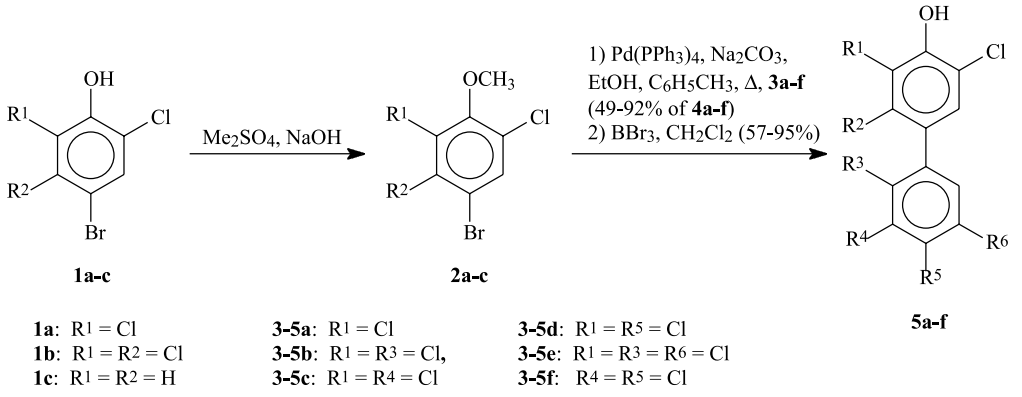
m.p. = 73–78°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.05 (br s, –OH, 1H), 6.85–6.90 (m, H-3), 7.19–7.24 (m, H-4,6), 7.32 (d, J = 8.4 Hz, d, J = 2.0 Hz, H-6′), 7.54 (d, J = 8.4 Hz, H-5′), 7.59 (d, J = 2.0 Hz, H-2′). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 117.56, 126.01, 127.33, 128.31, 129.44, 129.80, 130.93, 130.98, 132.44, 133.19, 136.04, 150.89. EI-MS m/z (relative intensity, %): 272 (100, $C_{12}H_7Cl_3O^{\cdot+}$), 236 (11, M–HCl), 202 (84, M–Cl_2), 173 (15, M-99), 139 (13, M-133). IR [cm^{-1}]: 3309 (br, ν (OH)), 1465, 1366, 1205, 811. Elemental analysis calcd. for $C_{12}H_7Cl_3O$: C 52.69, H 2.58; found: C 52.73, H 2.64.

2.24. 3′,5,5′-Trichloro-2-hydroxybiphenyl (7b)

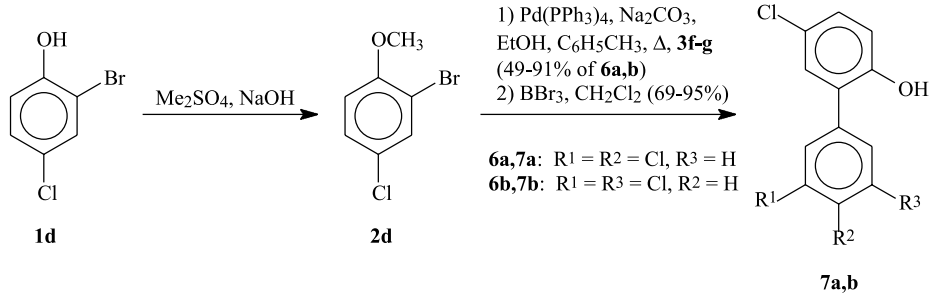
m.p. = 96–97°C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.02 (br s, –OH, 1H), 6.86–6.90 (m, H-3), 7.21–7.27 (m, H-4,6), 7.37–7.41 (m, H-2′,4′,6′). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 117.62, 125.98, 127.10, 127.52 (2 × C), 128.09, 129.64, 129.83, 135.46, 139.13, 150.97. EI-MS m/z (relative intensity, %): 272 (100, $C_{12}H_7Cl_3O^{\cdot+}$), 236 (13, M–HCl), 202 (83, M–Cl_2), 173 (16, M-99), 139 (13, M-133). IR [cm^{-1}]: 3565 (ν (OH)), 1559, 849, 810, 689. Elemental analysis calcd. for $C_{12}H_7Cl_3O$: C 52.69, H 2.58; found: C 52.70, H 2.55.

3. Results and discussion

All hydroxylated compounds **5** and **7** were synthesized by well-established methods as outlined in Schemes 1 and 2. The bromochlorophenols **1a–b** were prepared by bromination of the corresponding chlorophenol in glacial acetic acid. The chlorinated 2- or 4-bromo anisoles **2a–d** were obtained by methylation of



Scheme 1. Synthesis of 4-hydroxy PCBs ($R^n = H$ if not stated otherwise).



Scheme 2. Synthesis of 2-hydroxy PCBs.

the corresponding phenols **1**. Subsequent cross-coupling of an anisole **2** with a chlorinated arylboronic acid **3** gave the corresponding 2- or 4-methoxylated PCB congeners **4** and **6** in good to excellent yields. Some homo-coupling product of the arylboronic acid was formed despite vigorous exclusion of air. However, this byproduct could be easily removed by column chromatography on Alumina with *n*-hexanes as an eluent. As shown in Table 1, the hydroxylated PCB congeners **5** and **7** were obtained in good to excellent yields by demethylation with BBr_3 at ambient temperature followed by purification with column chromatography on silica gel.

The synthesis of 3-hydroxy PCB metabolites using this approach is not possible because the corresponding 3-bromoanisoles are not readily available. Furthermore we were unable to obtain PCB metabolites from 4-bromo-2,3,6-trichloroanisole **(2b)**. Although GS-MS analysis indicated the formation of the corresponding 4-methoxybiphenyls in good yields and purity, we observed an unknown impurity by ^{13}C NMR spectroscopy which could not be removed by column chromatography. We were also unable to purify the product after demethylation with borontribromide. Studies to overcome this problem are currently underway in our laboratory.

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